MNCs for 7d in RPMI-1640 + 10 IU IL-2. NKR expression (KIR2DS4, NKG2D, NKG2A, CD94, KIR3DL1, KIR3DL2, Nkp46), LAMP-1 receptor expression and NK cell phenotype ($CD56^{dim/bright}$ subsets) determined by flow cytometry. On Day 0, $CD3^-/56^+$ NK cell subset was 3.0 ± 1.3%. After 7d culture, NK cells increased to 71.7 ± 3.9%, compared to media alone (9.7 ± 2.4%) and WTK562 (42.6 ± 5.9%, $p < 0.01$). This represented a 35-fold or 3374 ± 385% increase of input NK cell number. CB Tcells were decreased compared to media alone and WTK562 (15.1 ± 1.7 vs 51 ± 7.1 vs 35.7 ± 2.4%, $p < 0.001$). $CD56^{bright}$ vs $CD56^{dim}$ subsets were increased (67 vs 33%, $p < 0.01$) following K562-mbIL15-41BBL stimulation. CB NK cells expressing KIR3DL1 were increased 10-fold following stimulation with K562-mbIL15-41BBL vs WTK562 ($p < 0.01$) and 5-fold increase in NK KIR2DS4 expression ($p < 0.05$), respectively. NK activation marker, CD107a was increased compared to WTK562 (51 ± 0.7 vs 32 ± 1.1%, $p < 0.05$). Since a standard cryopreserved UCB unit contains approximately 75×10^7 MNCs, by using the smaller aliquot (5 ml or 20%) 150×10^6 MNCs x 3.9% = 5.8×10^6NK cells, this method may yield 2.0×10^8 CB NK cells after 7d culture. This suggests that CBMNC can be expanded by K562-mbIL15-41BBL resulting in increased NK cell KIR expression (KIR2DS4, KIR3DL1) and NK activation (CD107a) with a decrease in T cells which may provide a means to enhance specific CB NK expansion for ACI in post UCBT setting.

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VIABILITY AND POTENCY OF HEMATOPOIETIC PROGENITOR CELLS AFTER PROLONGED CRYOPRESERVATION AT -80 °C

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Hematopoietic progenitor cell (HPC) products cryopreserved in the liquid or vapor phase of liquid nitrogen (LN) remain viable and potent for years. Cryopreserved HPC products when stored at -80 °C in mechanical freezers are known to remain viable and potent for up to 180 days. We demonstrated viability and potency of HPC products stored at -80 °C for up to 12 years. Products were cryopreserved in 6% hydroxyethyl starch, 5% DMSO and 4% human serum albumin using non-controlled rate freezing.

Thawed products were tested for viability and potency respectively by trypan blue dye exclusion and hematopoietic progenitor cell colony assay (CFU-GM, BFU-E, CFU-GEMM). All culture assays used MethoCult® GF^+ H4445 (STEMCELL Technologies, Vancouver, BC, Canada).

We thawed 20 HPC products collected from mobilized peripheral blood. The products were stored between 7 and 12 years at -80 °C. In 19 cases, trypan blue dye exclusion yielded an average 72% viability (range: 50%-89%). HPC colony assay yielded average progenitor frequencies of: CFU-GM 159 [range: 15-600]; BFU-E 116 [range: 2-388] and CFU-GEMM 21 [range: 2-68] all per 10^5 viable cells plated. The average values did not differ from control values of CFU-GM 111, BFU-E 121 and CFU-GEMM 23. Two products were infused into their donors after myeloablative conditioning, UPN 947 and UPN 1000. The products were stored for over 9 years. The products from UPN 947/UPN 1000 gave 73%/65% viability by trypan blue dye exclusion, CFU-GM 347/508, BFU-E 135/344, CFU-GEMM 12/48 per 10^5 viable cells, ANC500 day + 14/day + 11 and PLT50 day + 15/day + 24.

We conclude that viability and potency of these HPC products flash-frozen and stored at -80 °C in pentastarch is maintained for up to 12 years. In two cases engraftment was achieved with long-term cryopreserved products. The data suggest that prolonged cryopreservation of HPC products using LN may not be necessary to preserve product potency.

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DEVELOPMENT OF THE "MARROWMINER": A NOVEL, MINIMALLY INVASIVE DEVICE OF FOR THE HARVEST OF BONE MARROW. FROM BENCHTOP, TO ANIMAL STUDIES, THROUGH FDA APPROVAL AND HUMAN EVALUATION

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Bone marrow (BM) is the traditional graft source in hematopoietic stem cell transplantation. An increasing body of work suggests that use of BM grafts may show long-term advantages over mobilized PBSC in some alloegeneic transplant settings, resulting in significantly less cGVHD, morbidity and improved survival. Traditional OR based BM harvest methods however remain crude, labor and resource intensive, generally requiring full general anesthesia, 2 transplant clinicians, 100+ serial small volume needle aspirates, and result in grafts highly diluted by peripheral blood. Improved harvest methods are needed.

To address the need to develop improved methods for harvest, The "MarrowMiner" (MM) was conceived. The MarrowMiner (MM), is a novel, now FDA cleared & CE-Marked device developed for the minimally invasive harvest of BM to enable the rapid, convenient, outpatient harvest of large quantities of BM via a single marrow entry site, under local anesthesia. A powered, rotating FlexShaft enables the clinician to access a broad area of the ileac marrow space through a single cortical bone entry site trocar. The FlexShaft moves through the cancellous bone, without puncturing the cortical wall, while aspirating rich marrow under negative pressure into a closed container.

Preclinical trails in porcine models compared the marrow harvested with the MM to standard 6-hole harvest in the opposite ileac. This 6 pig study revealed a significantly richer bone marrow with >9 fold Colony Forming (CFU-F) per ml compared to standard harvest. Following FDA approval, successful 'First in Man' studies were conducted demonstrating safety and efficacy in patients having marrow harvested for regenerative medicine studies.

A 21 patient trial comparing the MM to standard harvest. The MARVELOUS (MARrowMiner VErsus standard ILeac bOne marrow pUncture and aSpiration)) study found that MM aspirate (performed under local anesthesia) had a greater average TNC count/ml compared to standard marrow harvest in the same patient, with equivalent viability. Higher numbers of %ALDH+, CD34+, phenotypic MSC) were obtained by MM.

The novel MM system demonstrated safety and efficacy in clinical use. An ongoing trial is evaluating the MM in the BMT setting, and a commercial version of the device is nearing production. The MM device device may benefit ease of harvest for donors, clinicians and could ultimately lead to better outcomes for transplant recipients.

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DETECTION AND EX VIVO EXPANSION OF DORMANT ANTI-VIRAL CTL PRECURSORS ISOLATED FROM RECIPIENTS OF UNRELATED UMBILICAL CORD BLOOD TRANSPLANT (UCBT)

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Viral infections are commonly detected within weeks after UCBT that could trigger *in vivo* priming of the infused naïve T cells. This hypothesis is supported by the observation that infections can resolve without antiviral therapy.

Objectives: In this study we focused on two potentially fatal viruses, CMV and adenovirus to assess the acquisition and evolution of antigen-specific immunity. We hypothesized that 1) threshold numbers of CTL precursors can be identified for each virus that may influence outcome 2) CTL precursors may reside below the level of detection during GVHD prophylaxis, however, ex vivo restimulation could expand them to measurable frequencies.

Methods: Monocytes infected with an adenovirus vector carrying the CMV pp65 transgene (Ad5f35pp65) were used as APC in the presence of IL7. Antiviral CTLp was quantitated by ELISPOT with computerized enumeration of IFNg producing spot forming cells (SFC) in response to peptide pools spanning dominant viral antigens: hexon and penton for adenovirus, pp65, EI-1 for CMV.

Results: 8 infected patients at a median age of 9.9 years have been studied. Blood was drawn at a median of 70 days (range 34-470). Only 1 of 3 patients with adenovirus infection (stool x1, urine x1, respiratory tract x 1) had associated viremia while all five (5) patients with CMV had viremia. Analyzing freshly drawn peripheral blood 1 of

3 adeno patients had detectable hexon-specific response (11 SFC/1×10e5 cells), however, none of the 5 CMV+ patients had any detectable SFC. However, after 9-12 days of stimulation we could enumerate CTLp in 7/8 patients. For adenovirus, the median hexon-specific response was 52 SFC/1×10e5 SFC (range 20-90), and the penton-specific CTLp frequency was 13 SFC/1×10e5 (1 – 25). The median CMV pp65-specific response was 6.7 SFC/1×10e5 (0-14). No responses were recorded against CMV EI-1 demonstrating the critical role of antigenic restimulation by the Ad5f35pp65 construct lacking IE. Smilarly in the adeno+/CMV- patients we detected SFC solely against hexon and/or penton. All patients are surviving at a median of 220 days after transplant (range 81-532).

Conclusion: To our knowledge these are the first data demonstrating that dormant virus-specific CTLp are present already in the first 2-3 months in most adenovirus or CMV infected UCBT recipients (7/8 in this dataset). Although these rare events are below detection by standard ELISPOT the feasibility of their ex vivo expansion may lead to adoptive therapies.

GVH/GVL

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COMMON COLD VIRUSES EARLY AFTER HSCT ARE ASSOCIATED WITH LIFE THREATENING ALLOIMMUNE LUNG SYNDROMES

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In lung transplantation, early infection (<100 d) with a common respiratory virus (RV) is associated with acute or chronic rejection, presenting as Bronchiolitis Obliterans (BO). After hematopoietic stem cell transplantation (HSCT) alloimmune lung syndromes (allo-LS), including Idiopathic Pneumonia Syndrome (IPS; acute) and BO (chronic), also occur, but the role of RV is unclear. In this prospective cohort study we analyzed the influence of common RV early (< 100 days) after HSCT, on the development of allo-LS and survival. 110 paediatric patients with a median age of 5 years (2 mths – 21years), were included. They received a transplant (56 matched, 54 mismatched; 33 cord blood, 77 bone marrow; 33 family, 77 unrelated) for malignant (56) and non-malignant (54) disease, after a TBI (33) or chemotherapy based (77) conditioning regimen. In 50% of patients a RV infection occurred, at a median of day +16 (range -7 to 100). RV was proven by qPCR on nasopharyngeal aspirate: Rhinovirus was found most frequent (28), followed by Parainfluenzavirus1-3, Coronavirus, Influenza A virus and Adenovirus. Clinical symptoms were mild, and all patients recovered spontaneously, despite the fact that the RV remained present for months in all patients. After a period without symptoms of at least 2 weeks, new respiratory symptoms occurred in about 50% of the RV positive patients. Based on additional examinations including negative cultures (other than RV), radiology and pulmonary function tests, 30 patients (27.2%) were diagnosed with allo-LS: 18 IPS (16.4%) and 12 BO (10.9%), after a median time of 8 weeks (2-26). Multivariable analysis showed that RV infection is an important predictor for allo-LS ($p < 0.0001$). There was no difference between Rhinovirus versus all others. Acute Graft-versus-Host Disease (aGVHD), occurring at a median of 4 weeks (2-15), had a protective effect on the development of allo-LS ($p = 0.004$), most likely due to higher and prolonged immunesuppression in these patients. Overall survival was 73%; in the allo-LS group this was only 53%. In multivariable analysis allo-LS was the only predictor for mortality ($p = 0.04$).

In conclusion, early presence of RV is a predictor for the development of allo-LS. We hypothesise that infection with a common cold virus makes the lung a target for alloimmunity, leading to life threatening lung disease. Paradoxically, prolonged immune suppression, despite the local viral infection, protected against the development of allo-LS.

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RAPID RECONSTITUTION OF THE REGULATORY T CELL COMPARTMENT AFTER HIGH DOSE CYCLOPHOSPHAMIDE IMMUNOSUPPRESSION PREVENTS THE DEVELOPMENT OF GVHD AFTER ALLOGENEIC BMT

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Graft-versus-host disease (GVHD) is a life-threatening complication of allogeneic bone marrow transplantation (BMT). Although the development of immunosuppressive regimens has reduced the incidence and severity of this post transplant complication, GVHD continues to be a significant clinical problem. There is also significant toxicity associated with the multi-agent immunosuppressive regimens. Recent studies revealed that high dose cyclophosphamide (Cy, 50 mg/kg) was effective at reducing the incidence of grades II-IV GVHD when administered as a single agent on days 3 and 4 following HLA matched related and unrelated BMT. The incidence of chronic GVHD was also reduced to 10%. The present studies evaluated the reconstitution of the CD4 + CD25 + Foxp3+ regulatory T cell compartment in patients treated with single agent high dose Cy. Peripheral blood lymphocytes (50 patients) were harvested on days 30, 45 and 60 post transplant and evaluated for Foxp3, IL-2 and IFN-γ mRNA transcript levels by real time PCR and by enumeration of CD4 + CD25 + Foxp3 + T cells by flow cytometry. The results reveal that there was an inverse correlation of Foxp3 mRNA transcripts with the incidence of grades II-IV GVHD. Levels of Foxp3 mRNA transcripts in patients who did not develop GVHD were >100 fold greater than the levels detected in patients who developed GVHD. Interestingly, mRNA transcript levels of IL-2 and IFN-γ were significantly increased (>100-1000 fold) in patients developing acute GVHD. Additional studies revealed that CD4 + CD25 + Foxp3 + T cells enumerated flow cytometrically in a subset of patients (16) were also detected at significant levels (30-50 cells/ul) as early as day 30 in patients who did not develop GVHD. Comparatively, the number of CD4 + CD25 + Foxp3 + T cells in patients who developed acute GVHD was <10 cells/ul and remained persistently low even at day 60. The peripheral blood mononuclear cells were also assessed for T cell recombinant excision circles (TRECs) as a marker for recent thymic emigrants. Surprisingly, TRECs were not detected at day 30. Taken together, these results suggest that there is a rapid recovery of CD4 + CD25 + Foxp3+ regulatory T cells in the majority of patients treated with high dose Cy which may prevent the development of GVHD. The absence of TRECs in these patients may suggest that the expansion of the regulatory compartment may be antigen driven from a mature T cell population or expanded by homeostatic mechanisms.

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PREVENTION OF ACUTE GVHD DURING MHC HAPLOIDENTICAL HSCT: EVALUATING THE EFFICACY OF T-CELL COSTIMULATION BLOCKADE USING A NOVEL RHESUS MACAQUE TRANSPLANT MODEL

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Introduction: Despite the inadequacy of current GvHD prophylaxis for MHC-mismatched HSCT, the introduction of novel therapeutics has been slow, likely due to the lack of a suitable translational model to rigorously test emerging therapies. Here we describe a novel Rhesus macaque model, which has allowed us to evaluate mechanisms controlling GvHD and its protection in a primate translational system.

Methods: Using microsatellite-based MHC haplotyping, we developed the first MHC-defined primate HSCT system. HSCT recipients, prepared with 8 Gy TBI, received either no immunosuppression or a costimulation blockade-based regimen which included CD28 blockade (abatacept), CD40 blockade (with the anti-CD40 antibody 3A8) and sirolimus after MHC haploidentical sibling HSCT. Recipients received a TNC dose of 9.3 +/- 2.7×10^8/kg and a CD3+ cell dose of 1.1 +/- 0.88 $\times$ 10^8/kg.

Results: Three control recipients (no immunosuppression) demonstrated rapid and complete donor engraftment, with T cell activation and proliferation occurring by Day 7, coincident with the onset of severe clinical GvHD. Flow cytometric analysis showed loss of